Measurement of Oxygen Metabolism using MRI. What Can and Cannot be Done?

Peter C.M. van Zijl
The Russel H. Morgan Department of Radiology and Radiological Sciences, Division of MR Research, Johns Hopkins University, Traylor Bldg. 217, 720 Rutland Ave., Baltimore, MD USA
F.M. Kirby Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD

In the last fifteen years, MRI has made much progress in providing non-invasive alternative methodologies for the quantification of physiological parameters in tissue, a field that previously was the exclusive domain of PET. It is now possible to assess cerebral blood flow (CBF [1-6]) and cerebral blood volume (CBV[6-10]) and blood oxygenation.[11-13] The ability to measure blood oxygenation using the BOLD[11-13] effect has stimulated the publication of several procedures for quantifying the cerebral metabolic rate of oxygen metabolism ($CMRO_2$). However, because of the inherent complexities of this effect, the determination of $CMRO_2$ by MRI is still a developing field. Here, after a brief description of the principles of BOLD contrast, an overview is provided of the existing literature on using this effect for quantification of oxygen metabolism.

A) Background on Hemodynamic measurements using MRI

The Blood oxygenation level dependent (BOLD) effect was suggested in 1990 by Ogawa et al.[11-13] The mechanism of BOLD signal changes is well understood to be related to changes in the concentration of deoxyhemoglobin, which acts as a paramagnetic contrast agent in capillary and venous blood.[14-18] When the blood oxygenation fraction (Y) is changed, the concentration of deoxyhemoglobin changes, which affects the transverse relaxation times T_2 and T_2^*. The relative concentration of paramagnetic deoxyhemoglobin ($[Hb]$) to total hemoglobin ($[Hb_{tot}]$) in venous blood is:[19]

$$\frac{[Hb]}{[Hb_{tot}]} = 1 - Y_v = 1 - Y_a + OEF \cdot Y_a \qquad [1]$$

Y_v and Y_a are the venous and arterial oxygen saturation fractions, respectively, and OEF is the oxygen extraction fraction, describing the ratio between oxygen consumption and delivery:

$$OEF = \frac{CMRO_2}{C_a \cdot CBF} = \frac{CMRO_2}{[Hb_{tot}] \cdot Y_a \cdot CBF} \qquad [2].$$

These equations directly show many of the contributions determining the BOLD effect. First, this effect directly reflects the so-called "coupling" between $CMRO_2$ and CBF. Second, the BOLD effect is very sensitive to total hemoglobin concentration ($[Hb_{tot}]$, expressed in mM), which is directly related to the hematocrit fraction (Hct). In most of the current fMRI literature, Hct is assumed to be constant during brain activation, but it is doubtful whether this is actually the case Third, the BOLD effect depends on the arterial oxygen saturation fraction, Y_a, a term that will become important during hypoxia. To avoid confusion, it needs to be mentioned that, in the early BOLD literature, Ogawa et al.[12,13] used the nomenclature oxygen extraction (O.E.), with which they meant $CMRO_2$ and not the oxygen extraction fraction (OEF). This should be kept in mind when reading this important pioneering work.

In most of the literature, Hct and arterial oxygenation are assumed constant, and the approximation

$$1 - Y_v = OEF = const \cdot CMRO_2 / CBF \qquad [3]$$

is used. When looking at changes in oxygenation of oxygen extraction fraction under the conditions of unchanged Hct, it can then be derived that changes in oxygen metabolism are given by:[20,21]

$$\left(1 + \frac{\Delta CMRO_2}{CMRO_2}\right) = \left(1 + \frac{\Delta CBF}{CBF}\right) \cdot \left(1 + \frac{\Delta OEF}{OEF}\right) = \left(1 + \frac{\Delta CBF}{CBF}\right) \cdot \left(1 - \frac{\Delta Y_v}{(1 - Y_v)}\right) \qquad [4]$$

This simple equation indicates that relative changes in $CMRO_2$ can be simply measured by performing experiments in which the relative changes in blood flow and blood oxygenation are determined. Of course, BOLD MRI does not measure oxygenation but water signal intensity or transverse relaxation time changes, which are a function of venous oxygenation level. In addition, an imaging experiment is not just a local measurement of water signal in the venous vessels, but a measurement of water intensity in a voxel containing multiple types of tissue. In the most optimum situation, the voxel would contain only parenchyma, i.e. grey matter and microvessels (arterioles, venules, and capillaries). In practice, voxels contain parenchyma, CSF, and often some parts of larger vessels, especial draining veins. To make matters worse, when CBF changes, the blood volume (CBV) generally also changes. Furthermore, BOLD effects do not occur only inside the blood vessels (intravascular BOLD) but also around them (extravascular BOLD) and the extravascular effects are different around the microvasculature and macrovasculature and differ for T_2 and T_2^*.[22,23] As if this is not enough, the Hct is also different between the microvasculature and large vessels. In order to be able to perform some quantification, most investigators have therefore resorted to trying to make some assumptions that seem reasonable under the particular experimental conditions and for the particular anatomy involved. Before discussing these assumptions, it is important to provide a brief overview about what is known about these relationships that govern the BOLD MRI signal in vivo.

1) Relationship between oxygenation changes and $T_2^{(*)}$ in blood (intravascular BOLD)

The exact quantitative relationships between Y_v and $R_2 = 1/T_2$ and $R_2^* = 1/T_2^*$ are still a topic of extensive study.[17,18,24-27] However, this dependence can be calibrated using experiments on isolated-blood phantoms under well-controlled physiological circumstances,[25,28] or, even using large blood vessels in vivo.[28-30] The simple hyperbolic equation commonly used for this intravascular BOLD calibration is:

$$R_2^* = A^* + C^* (1 - Y_v)^2 \qquad [5]$$

in which A^* and C^* are diamagnetic and paramagnetic rate constants that are dependent on the magnetic field strength, the hematocrit, and the particular pulse sequence and echo spacing used. Another term, describing a contribution from linear dependence on oxygenation ($B^*(1 - Y_v)$), is generally neglected. Values for these constants at multiple field strengths have been published (See Silvennoinen[25] and references therein). Several theories have also been suggested for describing these constants, e.g. in terms of the effects of water exchange through the erythrocyte membrane[16,17,19,30] and diffusion around it,[16,18,26] but irrespective of these theories, quite correct dependencies can always be measured in the form of a calibration. Actually, equation [5] has been used to determine OEF from oxygenation in large vessels,[28,29] even in draining veins from areas activated through visual stimulation.[30,31] Unfortunately, the spatial resolution of present MRI approaches at field strengths commonly used for human studies (1.5 T and 3.0 T) is still somewhat limited and localization inside brain vessels is only possible in larger veins, which are

the convolution of draining pial veins of many regions. Also, they again depend on the blood hematocrit, which is known to differ between the microvasculature and larger blood vessel.

2) Relationship between oxygenation changes and $T_2^{(*)}$ in tissue (extravascular BOLD)

Ogawa et al.[12,13], Yablonski and Haacke[32] and Weisskoff et al.[22,23] derived expressions for the extravascular BOLD effect. These theories are based on the dephasing of water in large field gradients caused by deoxyhemoglobin. Assuming a blood vessel can be approximated by a long cylinder, gradients around a random network of such cylinders can cause a frequency shift ($\delta\omega$):

$$\delta\omega = \gamma \cdot B_0 \cdot \frac{4}{3}\pi \cdot \Delta\chi_{deoxy} \cdot Hct \cdot (1 - Y_v) \qquad [6]$$

in which γ is the gyromagnetic ratio, B_0 the static magnetic field, and $\Delta\chi_{deoxy}$ the magnetic susceptibility difference between oxygenated and fully deoxygenated blood (~0.2ppm).[17,27] The estimated hematocrit in the microvasculature is about 85% of that in the large vessels. Two types of dephasing effects can occur due to the presence of these field differences, one from coherent dephasing (larger microvasculature) and one from incoherent dephasing due to diffusional motion (very small microvessels in the capillary network). The signal decay due to has an exponent that depends on the time scale:[32]

Larger microvessels:	Exponent ~ $CBV_v \cdot \delta\omega \cdot TE - 1$	$\delta\omega \cdot TE \geq 1.5$	[7a]
smaller microvessels:	Exponent ~ $0.3CBV_v \cdot (\delta\omega \cdot TE)^2$	$\delta\omega \cdot TE < 1.5$	[7b]

The first equation corresponds the so-called static dephasing regime, in which dephasing of water spins due to the presence of static magnetic field gradients around the vessels is much larger than the diffusional dephasing of the spins (i.e. large gradient, slow motion). This approximation, in which $R_2^{'} = CBV_v \cdot \delta\omega$, applies for somewhat larger vessels at longer echo times (~32ms at 1.5T). At higher magnetic field strength shorter TEs, where the gradients are becoming larger, proportionally shorter TEs can be used. Using simulations, others[22,23,33] et al found similar dependencies as a function of vessel size, showing that the static dephasing regime applies to most vessels except very small capillaries, where diffusional approaches need to be used.[33-35] When pure extravascular effects play a role, these dependencies result in the phenomenon that extravascular spin echo (SE) effects mainly localize around small vessels, while extravascular gradient echo (GRE) effects occur around both large and small vessel.[22,23,36] This is obviously not the case for intravascular SE and GRE effects, which occur in all capillaries and venous vessels (large and small).[37-40]

Ogawa et al.[12], when using static averaging derived an empirical equation for the dephasing contribution due to hemoglobin, namely $R_2^* \sim R_2^{'} = A \cdot \gamma B_0 / 1.32 = 2\pi\Delta\chi_{deoxy}\gamma B_0 / 1.32$. So the constant is about a factor of 3 too large for Ogawa. In his work he use a very small susceptibility difference (~0.10 ppm), which reduces the difference to less than 50%.

3) Relationship between CBV and CBF changes.

Even though there need not be a particular relationship between CBF and CBV, the BOLD fMRI literature has used the existence of an empirical relationship between these two hemodynamic parameters to reduce the number of independent parameters. This relationship is based on a paper by Grubb et al.,[41] who studied flow and volume effects in the monkey brain during variation in CO_2 level, which gave the following relationship:

$$CBV_1 = CBV_2 \cdot \left(\frac{CBF_1}{CBF_2} \right)^{\alpha} \qquad [8a]$$

There was a large data spread in these experiments that can be fit with many different functions. For the simple power function above and using a two-parameter fit, Grubb found $\alpha = 0.38$. Van Zijl et al.[19] later used $\alpha = 0.50$, which also fits Grubb's data well and which corresponds to the physical case of a random network of cylinders. This equation can be rewritten in terms of CBV and CBF changes as:

$$\frac{\Delta CBV}{CBV} = \left(\frac{\Delta CBF}{CBF} + 1 \right)^{\alpha} - 1 \qquad [8b]$$

B) Measurement of oxygen metabolism using MRI

It can be seen from the above information that determination of oxygen metabolism requires either the measurement of multiple hemodynamic parameters for multiple voxel components or the use of several simplifying experimental conditions or assumptions.

1) Determination of OEF in draining veins

As mentioned above, the most simple situation occurs when it is possible to measure relaxation rates in isolated blood vessels assuming a mono-exponential signal decay. Even this is not simple, as, for a single spin echo, the relaxation rate itself depends on TE and thus is inherently non-mono-exponential. However, it has been found to experimentally approach mono-exponential behavior when using a multi-echo so-called Carr-Purcell-Meiboom-Gill (CPMG) sequence, even though this may also not be the case due to the presence of slow exchange between the erythrocyte and plasma compartments (Springer), especially at higher magnetic fields. In this case, a calibration can be performed either in situ[28] or using a phantom[25] and the venous oxygenation during baseline activity, neuronal activation, or any other physiological change can be directly used to determine from the transverse relaxation rate using Eqn. [5]. It should be noticed that Oja, Silvennoinen, and Golay et al.[25,30,31] did not use equation [5] but assumed a simple exchange model for blood relaxation Eqn. [5]. Even though the correctness of such a model has not been demonstrated at all magnetic field strengths, the resulting parameters even though potentially without exact physical meaning can still be seen as arbitrary calibration constants as long as the model fits the data well. So the principle is similar to Eqn. [5].

When studying visual activation, Oja et al.[31] reported resting OEF of 0.30±0.06, corresponding to $Y_v = 0.69$. This is a bit higher than the usual saturation fraction of 0.61 in the veins. OEF changed to 0.20±0.06 during visual activation. The OEF reduction of 33% is close to that measured by Fox et al.[42]

2) OEF and CMRO2 determination in vivo under the assumption of BOLD being extravascular

As mentioned above, until the present, most people have assumed constant Hct during small physiological changes such as brain activation, which was used in the derivation of Eqn. [4].

A second assumption is the presence of mono-exponential signal decay, which is not correct, but may be the only attainable reasonable approach with the limited signal to noise in vivo at present. *As far as modeling is concerned, the most common assumption made by investigators in this field[10,20,21,43,44] is that the BOLD effect is predominantly extravascular and that therefore the BOLD change is linear in CBV.* Unfortunately, experimental data by Boxerman et al.,[36] Song et al.,[45] and Lu et al.[46] have shown that BOLD is mainly intravascular at 1.5T (about 50-70%) and to a large extent intravascular at 3T (about 30-35%), the fields at which most oxygen consumption measurements have been reported. This mismatch between assumption and reality, and the effect of field strength upon it, have to be kept clearly in mind when reading the relevant literature. At very high fields the venous relaxation rates become very small and, at longer TE,

there is little intravascular signal remaining in the veins. However, the tendency is to go to shorter TE at higher field and to use spin echoes, so it is unlikely that all intravascular signal can be removed from capillary regions even at fields as high as 9.4 T. also, during activation, the intravascular changes would be extra large due to the large values for A^* and especially C^* at such fields.[25]

2a: Use of static dephasing regime to determine CMRO2 without calibration

In 1997, Kim and Ugurbil[20] were the first to use a combined CBF/BOLD measurement to assess $CMRO_2$ changes. They employed Ogawa's equations to estimate relative $CMRO_2$ changes during visual activation under the assumption of a reasonable venous blood volume of 3% and a baseline Y_v 0f 0.54 (a bit low compared to normal venous levels of about 0.61). The resulting changes during visual stimulation at a field of 4T were found to be 43% in CBF and 5.4% in $CMRO_2$. This is smaller than expected and can probably be attributed to the A-factor used, which included the assumed very small $\Delta\chi_{deoxy}$.

In 2000, An and Lin[47] described an elegant approach in which tissue oxygen saturation and blood volume can be derived using Yablonskiy's theory and a determination of the difference between spin echo and gradient echo signal decays. After removing the R_2 effect from the signal the remaining curve was assumed to represent the MR signal dependence in the presence of static local magnetic field inhomogeneity. This signal was then fitted to determine $R_2^{'}$ and CBV_v, from which the venous tissue oxygenation was used to determine $Y_v = 0.58 \pm 0.02$, very close to the physiologically expected value. However, CBV_v was found to be close too 16%, much too large. In addition to potential partial volume contributions with large vessels, this could be due to the presence of static field inhomogeneities unrelated to Hb. Actually, it seemed that the effect of CBV and additional field inhomogeneities compensated each other, explaining the correct oxygenation level. In a very relevant follow-up paper,[10] these authors addressed this problem by also acquiring a field map and using this to correct the data. They now found very reasonable venous blood volumes (~2.6-3.1%) and very reasonable tissue OEF values (0.45-0.48). In other papers by this group, they combined their approach with CBF measurements to map absolute $CMRO_2$[48] and they addressed the effects of intravascular contributions.[49] These methods have shown potential for the use in imaging of acute ischemia.[50] When automated, this could become a very practical approach.

2a: Use of hypercapnia calibration to determine $CMRO_2$

Other authors have taking a semi-empirical semi-theoretical approach with an estimated coefficient for the oxygenation (or OEF) dependence of the relaxation time change during a physiological change such as brain activation. They have combined this with a calibration of this dependence using hypercapnia, where vascular dilatation is expected to occur without oxygenation changes. Two groups[21,43] have done this and they both use exponents α and β, but unfortunately they have different meaning, making the literature somewhat confusing. We will continue to use α as defined above as Grubb's constant and rename the constants for the second approach[43] as a* and b*.

- Approach of Davis et al.[21]

Davis et al derived an expression in which the effect of blood volume expansion without hemoglobin is inserted as a calibration constant (M), while the extravascular effect of magnetic field gradients around the vessel network is approximated from earlier simulations and measurements of this group for the BOLD effect showing a power dependence of about $\beta = 1.5$, i.e. somewhere in between that of Eqs. 7a and 7b. The relative BOLD change is then:

$$\frac{\Delta S}{S} = M\left\{1-\left(\frac{CMRO_2^{act}}{CMRO_2}\right)^{\beta}\left(\frac{CBF^{act}}{CBF}\right)^{\alpha-\beta}\right\} \qquad [9]$$

Using this approach for studying visual activation, they found M = 0.22 ± 0.03 and determined that the change in oxygen metabolism was about half of that of the blood flow change for the most potent visual stimulation (25% versus 48%).

- Approach of Kim et al.[43]
Based on Ogawa's equations, Kim et al.[43] derived the following approximation for the BOLD gradient echo signal changes:

$$\Delta R_2^* = -\frac{\Delta S}{S}\cdot\frac{1}{TE} = -a^*\left[\frac{\Delta Y_v}{1-Y_v} - b^*\frac{\Delta CBV_v}{CBV_v}\right] \qquad [10]$$

When experimentally determining b*, they found it to be only a few percent (-0.07), which is unlikely in view of expected CBV contributions to the BOLD effect. Therefore they used both b* = 0 (method 1) and b* = 1 (Method 2) and, using hypercapnia, determined the accompanying a* values at 1.5T (1.62^{-1} and $3.14s^{-1}$, resp). The determined oxygen metabolism changes during visual stimulation (8Hz, black&white checkerboard) were about 30±19% and 16±8%, with a flow change of about 44±9%. In 2003, Feng et al.[44] used this same approach for event-related visual stimulation (1s). The assumed b* = 1 and a* proportional to the field, leading to a* = 3.98 at 1.9T. They subsequently determined average oxygen metabolism changes of about 10±4% for CBF changes of 17±4%.

3) Determination of CMRO2 in vivo under the condition of BOLD being extravascular
Recently, a new MRI technology was developed in which CBV changes can be measured through nulling of the blood signal.[9] The echo time dependence in this experiment depends on the BOLD effect, but because the blood signal is nulled, this approach directly measures only the extravascular BOLD component.[46] Furthermore, because this approach is localized in areas where the CBV changes occur, the effect occurs predominantly in the microvasculature. By determining absolute extravascular relaxation rates, the extravascular ΔR_2^* could be directly determined. Assuming that 70% of the microvasculature is venous and a baseline total blood volume of 4.7% (CBV_v= 3.29%), the OEF during activation could be determined by assuming a baseline oxygenation level of 61% (OEF = 0.38). The activated OEF was determined to be 0.22 during 8Hz yellow-blue checker-board stimulation.[46]

4) Determination of $\overline{CMRO_2}$ in vivo using combined intra/extravascular models
When studying the BOLD effect without blood nulling, it is necessary to have a multi-compartment model for the parenchyma.[19,51,52] In a recent paper, Lu et al.[53] combined CBF (arterial spin labeling or ASL), CBV (VAscular Space Occupancy or VASO), and BOLD imaging in a study of visual activation. When selecting only voxels overlapping between the three methods, the BOLD response was totally different, with much larger effects both during stimulation and after stimulation (post-stimulus undershoot). Because the VASO method is based on microvascular changes, it was concluded that this is the true parenchymal BOLD appearance. A model for parenchyma needs to include arteriolar (a), capillary (c), venular (v), and tissue (t) components. To simplify things, they assumed capillary can be seen as part arteriolar and part venular, and used a two-compartment microvascular model (30% a, 70% v). When doing so, the MRI signal is:[53]

$$S \sim \sum_i x_i \cdot M_i \cdot e^{-R2i^*} \qquad [11]$$

in which M_i describes the dependence on repetition (TR) and flip angle (FA), and x_i is the water fraction, that relates to blood volume and tissue fractions via the water density. Using the static dephasing equations for the tissue relaxation (Eq. 7a) and the intravascular calibration (Eq. 5) for intravascular BOLD , they were able to determine OEF and CMRO2 changes upon simple assumption of baseline total CBV = 0.047, baseline Yv = 0.61, and $\Delta\chi_{deoxy}$ = 0.31ppm. The derived equation for $\Delta S/S$ was quite exact, including also arteriolar changes, but there was a general assumption that the blood-volume changes in arterioles and venules follow the same expansion, which may not be true. However, as the arteriolar BOLD effect is small, this is not a major issue. The total equations was:

$$\frac{\Delta S}{S} = \frac{0.3\Delta x M_a e^{-R2a^{TE}} + 0.7M_v(x^{act}e^{-R2v^{*,act}TE} - x^{rest}e^{-R2v^{*}TE}) + M_t e^{-R2,t^{TE}}[e^{-\Delta R2,t^{TE}}(1-x^{act}) - (1-x)]}{0.3xM_a e^{-R2a^{TE}} + 0.7xM_v e^{-R2v^{TE}} + (1-x)M_t e^{-R2,t^{TE}}}$$

in which the water fraction change is directly related to the VASO signal change. The change in CBF was about 65-75%, while the change in $CMRO_2$ was about 20%.

C) Conclusions

As can be judged from above, the last decade has shown a lot of activity in making the determination of $CMRO_2$ part of the MRI quantification package. Even though such a determination is possible using the BOLD effect, great care has to be taken in doing so. Most methods include a variety of assumptions that may be more or less valid, depending on the MRI sequence parameters and spatial resolutions used. Also, the results may vary with field strength. This is reflected in the above results that show that, when studying visual activation in humans, the resulting $CMRO_2$ changes measured vary from a few percent to as high as 30%. Probably the only thing that can be concluded for sure is that $CMRO_2$ and CBF changes are mismatched during activation, that is, at the spatial resolution presently used.

What can be concluded about the methods is that models are becoming more mature and that, with the recent availability of approaches to determine CBV and thus water fractions, it should be possible to use relatively straightforward equations with limited assumptions to determine this important physiological parameter.

1. Alsop, D.C. & Detre, J.A. Multisection cerebral blood flow MR imaging with continuous arterial spin labeling. *Radiology* **208**, 410-416 (1998).
2. Detre, J.A., Leigh, J.S., Williams, D.S. & Koretsky, A.P. Perfusion imaging. *Magn Reson Med* **23**, 37-45 (1992).
3. Kim, S.-G. Quantification of relative cerebral blood flow change by flow-sensitive alternating inversion recovery (FAIR) technique: application to functional mapping. *Magn. Reson. Med.* **34**, 293-301 (1995).
4. Kwong, K.K. et al. MR perfusion studies with T1-weighted echo planar imaging. *Magn Reson Med* **34**, 878-87 (1995).
5. Ostergaard, L. et al. Cerebral blood flow measurements by magnetic resonance imaging bolus tracking: comparison with [(15)O]H2O positron emission tomography in humans. *J Cereb Blood Flow Metab* **18**, 935-40 (1998).
6. Ostergaard, L. et al. Absolute cerebral blood flow and blood volume measured by magnetic resonance imaging bolus tracking: comparison with positron emission tomography values. *J Cereb Blood Flow Metab* **18**, 425-32 (1998).
7. Rosen, B.R. & et al. Perfusion imaging by nuclear magnetic resonance. *Magn Reson Q.* **5**, 263-81. Review. No abstract available. (1989).
8. Rosen, B.R. et al. Susceptibility contrast imaging of cerebral blood volume: human experience. *Magn Reson Med* **22**, 293-9; discussion 300-3 (1991).
9. Lu, H., Golay, X., Pekar, J.J. & Van Zijl, P.C. Functional magnetic resonance imaging based on changes in vascular space occupancy. *Magn Reson Med* **50**, 263-74 (2003).
10. An, H. & Lin, W. Cerebral oxygen extraction fraction and cerebral venous blood volume measurements using MRI: effects of magnetic field variation. *Magn Reson Med* **47**, 958-66 (2002).
11. Ogawa, S., Lee, T.M., Kay, A.R. & Tank, D.W. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc. Natl. Acad. Sci. USA* **87**, 9868 - 9872 (1990).
12. Ogawa, S. et al. Functional brain mapping by blood oxygenation level-dependent contrast magnetic resonance imaging: a comparison of signal characteristics with a biophysical model. *Biophys. J.* **64**, 803-812 (1993).

13. Ogawa, S., Lee, T.M. & Barrere, B. The sensitivity of magnetic resonance image signals of a rat brain to changes in the cerebral venous blood oxygenation. *Magn. Reson. Med.* **29**, 205-210 (1993).
14. Eisenstadt, M. & Fabry, M.E. NMR relaxation of the hemoglobin-water proton spin system in red blood cells. *J. Magn. Reson.* **29**, 591-597 (1978).
15. Fabry, M.E. & San George, R.C. Effect of magnetic susceptibility on nuclear magnetic resonance signals arising from red cells. *Biochemistry* **22**, 4119-4125 (1983).
16. Thulborn, K.R., Waterton, J.C., Matthews, P.M. & Radda, G.K. Oxygenation dependence of the transverse relaxation time of water protons in whole blood at high field. *Biochim. Biophys. Acta* **714**, 265 - 270 (1982).
17. Bryant, R.G., Marill, K., Blackmore, C. & Francis, C. Magnetic relaxation in blood and blood clots. *Magn. Reson. Med.* **13**, 133-144 (1990).
18. Gillis, P., Peto, S., Moiny, F., Mispelter, J. & Cuenod, C.A. Proton transverse nuclear magnetic relaxation in oxidized blood: a numerical approach. *Magn Reson Med* **33**, 93-100. (1995).
19. van Zijl, P.C. et al. Quantitative assessment of blood flow, blood volume and blood oxygenation effects in functional magnetic resonance imaging. *Nat Med* **4**, 159-167 (1998).
20. Kim, S.-G. & Ugurbil, K. Comparison of blood oxygenation and cerebral blood flow effects in fMRI: Estimation of relative oxygen consumption change. *Magn. Reson. Med.* **38**, 59-65 (1997).
21. Davis, T.L., Kwong, K.K., Weisskoff, R.M. & Rosen, B.R. Calibrated functional MRI: mapping the dynamics of oxidative metabolism. *Proc Natl Acad Sci U S A* **95**, 1834-9 (1998).
22. Boxerman, J.L., Hamberg, L.M., Rosen, B.R. & Weisskoff, R.M. MR contrast due to intravascular magnetic susceptibility perturbations. *Magn Reson Med* **34**, 555-66 (1995).
23. Weisskoff, R.M., Zuo, C.S., Boxerman, J.L. & Rosen, B.R. Microscopic susceptibility variation and transverse relaxation: theory and experiment. *Magn Reson Med* **31**, 601-10 (1994).
24. Spees, W.M., Yablonskiy, D.A., Oswood, M.C. & Ackerman, J.J. Water proton MR properties of human blood at 1.5 Tesla: magnetic susceptibility, T_1, T_2, T_2*, and non-Lorentzian signal behavior. *Magn Reson Med* **45**, 533-542. (2001).
25. Silvennoinen, M.J., Clingman, C.S., Golay, X., Kauppinen, R.A. & Van Zijl, P.C. Comparison of the dependence of blood R2 and R2* on oxygen saturation at 1.5 and 4.7 Tesla. *Magn Reson Med* **49**, 47-60 (2003).
26. Jensen, J.H. & Chandra, R. NMR relaxation in tissues with weak magnetic inhomogeneities. *Magn Reson Med* **44**, 144-56 (2000).
27. Brooks, R.A. & Di Chiro, G. Magnetic resonance imaging of stationary blood: a review. *Med Phys* **14**, 903-913. (1987).
28. Wright, G.A., Hu, B.S. & Macovski, A. Estimating oxygen saturation of blood in vivo with MR imaging at 1.5T. *J Magn Reson Imag* **1**, 275-283 (1991).
29. Foltz, W.D., Merchant, N., Downar, E., Stainsby, J.A. & Wright, G.A. Coronary venous oximetry using MRI. *Magn Reson Med* **42**, 837-48 (1999).
30. Golay, X. et al. Measurement of tissue oxygen extraction ratios from venous blood T_2: Increased precision and validation of principle. *Magn Reson Med* **46**, 282-291. (2001).
31. Oja, J.M., Gillen, J.S., Kauppinen, R.A., Kraut, M. & van Zijl, P.C. Determination of oxygen extraction ratios by magnetic resonance imaging. *J Cereb Blood Flow Metab* **19**, 1289-1295. (1999).
32. Yablonskiy, D.A. & Haacke, E.M. Theory of NMR signal behavior in magnetically inhomogeneous tissues: the static dephasing regime. *Magn Reson Med* **32**, 749-63 (1994).
33. Kiselev, V.G. & Posse, S. Analytical model of susceptibility-induced MR signal dephasing: effect of diffusion in a microvascular network. *Magn Reson Med* **41**, 499-509 (1999).
34. Sukstanskii, A.L., Ackerman, J.J. & Yablonskiy, D.A. Effects of barrier-induced nuclear spin magnetization inhomogeneities on diffusion-attenuated MR signal. *Magn Reson Med* **50**, 735-42 (2003).
35. Sukstanskii, A.L., Yablonskiy, D.A. & Ackerman, J.J. Effects of permeable boundaries on the diffusion-attenuated MR signal: insights from a one-dimensional model. *J Magn Reson* **170**, 56-66 (2004).
36. Boxerman, J.L. et al. The intravascular contribution to fMRI signal change: Monte Carlo modeling and diffusion-weighted studies in vivo. *Magn Reson Med* **34**, 4-10. (1995).
37. Hoogenraad, F.G.C. et al. In vivo measurement of changes in venous blood oxygenation with high resolution functional MRI at 0.95 Tesla by measuring changes in susceptibility and velocity. *Magn. Reson. Med.* **39**, 97-107 (1998).
38. Oja, J.M.E., Gillen, J.S., Kauppinen, R.A., Kraut, M. & van Zijl, P.C.M. Venous blood effects in spin echo fMRI of human brain. *Magn Reson Med* **42**, 617-627 (1999).
39. Reichenbach, J.R. et al. Theory and application of static field inhomogeneity effects in gradient-echo imaging. *J Magn Reson Imaging* **7**, 266-79 (1997).
40. Lai, S. et al. Identification of vascular structures as a major source of signal contrast in high resolution 2D and 3D functional activation imaging of the motor cortex at 1.5T: preliminary results. *Magn Reson Med* **30**, 387-92 (1993).
41. Grubb, R.L., Jr., Raichle, M.E., Eichling, J.O. & Ter-Pogossian, M.M. The effects of changes in P_aCO_2 on cerebral blood volume, blood flow, and vascular mean transit time. *Stroke* **5**, 630-639. (1974).
42. Fox, P.T., Raichle, M.E., Mintun, M.A. & Dence, C. Nonoxidative glucose consumption during focal physiologic neural activity. *Science* **241**, 462-464 (1988).
43. Kim, S.G., Rostrup, E., Larsson, H.B., Ogawa, S. & Paulson, O.B. Determination of relative CMRO2 from CBF and BOLD changes: significant increase of oxygen consumption rate during visual stimulation. *Magn Reson Med* **41**, 1152-61 (1999).
44. Feng, C.M., Liu, H.L., Fox, P.T. & Gao, J.H. Dynamic changes in the cerebral metabolic rate of O2 and oxygen extraction ratio in event-related functional MRI. *Neuroimage* **18**, 257-62 (2003).
45. Song, A.W., Wong, E.C., Tan, S.G. & Hyde, J.S. Diffusion weighted fMRI at 1.5 T. *Magn Reson Med* **35**, 155-158. (1996).
46. Lu, H. & van Zijl, P.C. Experimental measurement of extravascular parenchymal BOLD effects and tissue oxygen extraction fractions using multi-echo VASO fMRI at 1.5 and 3.0 T. *Magn Reson Med* **53**, 808-16 (2005).
47. An, H. & Lin, W. Quantitative measurements of cerebral blood oxygen saturation using magnetic resonance imaging. *J Cereb Blood Flow Metab* **20**, 1225-36 (2000).
48. An, H., Lin, W., Celik, A. & Lee, Y.Z. Quantitative measurements of cerebral metabolic rate of oxygen utilization using MRI: a volunteer study. *NMR Biomed* **14**, 441-7 (2001).
49. An, H. & Lin, W. Impact of intravascular signal on quantitative measures of cerebral oxygen extraction and blood volume under normo- and hypercapnic conditions using an asymmetric spin echo approach. *Magn Reson Med* **50**, 708-16 (2003).
50. Lin, W. et al. Quantitative measurements of cerebral blood flow in patients with unilateral carotid artery occlusion: a PET and MR study. *J Magn Reson Imaging* **14**, 659-67 (2001).
51. Kennan, R.P., Scanley, B.E., Innis, R.B. & Gore, J.C. Physiological basis for BOLD MR signal changes due to neuronal stimulation: separation of blood volume and magnetic susceptibility effects. *Magn Reson Med* **40**, 840-6 (1998).
52. Kennan, R.P., Scanley, B.E. & Gore, J.C. Physiologic basis for BOLD MR signal changes due to hypoxia/hyperoxia: separation of blood volume and magnetic susceptibility effects. *Magn Reson Med* **37**, 953-6 (1997).
53. Lu, H., Golay, X., Pekar, J.J. & Van Zijl, P.C. Sustained poststimulus elevation in cerebral oxygen utilization after vascular recovery. *J Cereb Blood Flow Metab* **24**, 764-70 (2004).